

Supplementary information

Supplementary Table1. The primers used in this study.

Primer	Sequence	Use
sgRNA-HXGPRT-F	5'-GATCGCTCCAACAGCTTGAAG TTT TAGAGCTAGAAATAGC-3'	To construct the HXGPRT specific CRISPR plasmid
sgRNA-OMPDC-F	5'-GAAGACTCTCCATCCACGACG TTT TAGAGCTAGAAATAGC-3'	To construct the OMPDC specific CRISPR plasmid
sgRNA-R	5'-AACTTGACATCCCCATTTAC-3'	To construct the HXGPRT and OMPDC specific CRISPR plasmid
HX-UPRT-F	5'-TATTCCTTTTTTCGTCGGACCTT TCCACAGGGCTTCTAAAGAT CAGCACGAAACCTTGCA-3'	Amplification of 5'-homology of UPRT
HX-UPRT-R	5'-TCCGCGATTCCGTCAGCGGTCT GTCAAAAAAAGTAGAGACG TGGATCCCCCTCCACCGCG-3'	Amplification of 3'-homology of UPRT
UPRT-5'utr-F	5'-GAGCAACTTACAAGGACAAGG-3'	To construct the homology of UPRT3'utr-5'utr
UPRT-3'utr5'utr-R	5'-CCGTCAGCGGTCTTTTAGAAGC CCTGTGGA-3'	To construct the homology of UPRT3'utr-5'utr
UPRT-5'utr3'utr F	5'-CAGGGCTTCTAAAAGACCGCTG ACGGAATC-3'	To construct the homology of UPRT3'utr-5'utr
UPRT-3'utr R	5'-TCTTCACATTGCTTCAACAA-3'	To construct the homology of UPRT3'utr-5'utr
HX-OMPDC-F	5'-GACATTTTTCCGTGACAATTCCA AAGAACTGTCAAATAAGGAT CAGCACGAAACCTTGCA-3'	Amplification of 5'-homology of OMPDC
HX-OMPDC-R	5'-ATGTAGACCAGAGTCACGATAGT GTTTCGCTTTTCAGCACGGTG GATCCCCCTCCACCGCG-3'	Amplification of 3'-homology of OMPDC
UPRT-F1	5'-GCGGTAAGACGAGGGCCATATC-3'	PCR2 of $\Delta ku80\Delta hxgprt$

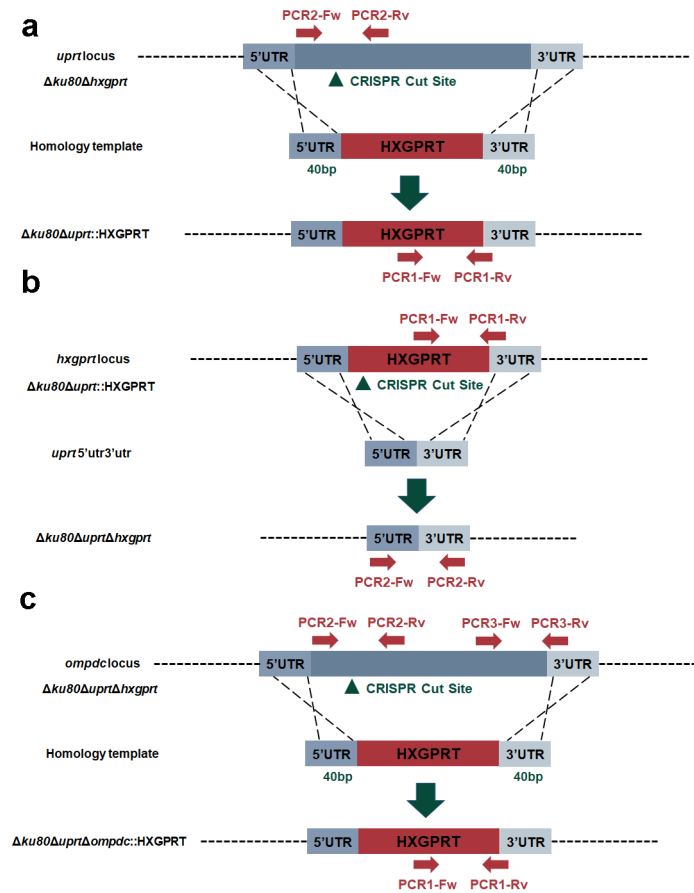
		and $\Delta uprt::HXGPRT$
UPRT-R1	5'-GAAGATTATTCCGCACCTTAC-3'	PCR2 of $\Delta ku80\Delta hxp rt$ and $\Delta uprt::HXGPRT$
UPRT-F2	5'-GTTGTGTCTGTGGAGGAAG-3'	PCR2 of $\Delta uprt::HXGPRT$ and $\Delta uprt\Delta hxp rt$
UPRT-R2	5'-ATGTCGTCGCAGTAGTGA-3'	PCR2 of $\Delta uprt::HXGPRT$ and $\Delta uprt\Delta hxp rt$
529bp-F	5'-CGCTGCAGGGAGGAAGACGA AAGTTG-3'	PCR3 of $\Delta uprt::HXGPRT$ and $\Delta uprt\Delta hxp rt$
529bp-R	5'-CGCTGCAGACACAGTGCATCT GGATT-3'	PCR3 of $\Delta uprt::HXGPRT$ and $\Delta uprt\Delta hxp rt$
HXGPRT-F	5'-GAGCACTATGTCCGCCTGAA-3'	PCR1 of $\Delta uprt::HXGPRT$, $\Delta uprt\Delta hxp rt$ and $\Delta ompdc\Delta uprt::HXGPRT$
HXGPRT-R	5'-TGTACACTTAGGTGTCGCGG-3'	PCR1 of $\Delta uprt::HXGPRT$, $\Delta uprt\Delta hxp rt$ and $\Delta ompdc\Delta uprt::HXGPRT$
OMPDC-F1	5'-GATAAGTCGCACGCCAGAGGAG-3'	PCR2 of $\Delta uprt\Delta hxp rt$ and $\Delta ompdc\Delta uprt::HXGPRT$
OMPDC-R1	5'-TCGCACGCATCTCAGATTGTCA-3'	PCR2 of $\Delta uprt\Delta hxp rt$ and $\Delta ompdc\Delta uprt::HXGPRT$
OMPDC-F2	5'-GGTTGTCCAGGCTGCTGTGAAT-3'	PCR3 of $\Delta uprt\Delta hxp rt$ and $\Delta ompdc\Delta uprt::HXGPRT$
OMPDC-R2	5'-CTATCCGTGGTGA CTGCGAAGG-3'	PCR3 of $\Delta uprt\Delta hxp rt$ and $\Delta ompdc\Delta uprt::HXGPRT$
RT-OMPDC-F	5'-CGCATCATCAGGGAACTCTTC-3'	qRT-PCR of $\Delta ompdc\Delta uprt::HXGPRT$
RT-OMPDC-R	5'-TAGGAACATCGTCGGGAATCAG-3'	qRT-PCR of $\Delta ompdc\Delta uprt::HXGPRT$
RT-UPRT-F	5'-TTTCCCAATGTGGTGCTC-3'	qRT-PCR of $\Delta ompdc\Delta uprt::HXGPRT$
RT-UPRT-R	5'-GCTTCTTCGATGAGGAGG-3'	qRT-PCR of $\Delta ompdc\Delta uprt::HXGPRT$

RT-GAPDH-F	5'-ATTTTGCTTGGGATTTCGAGGA-3'	qRT-PCR of GAPDH
RT-GAPDH-R	5'-TGCAGGGTAACGATCAAAAAATG-3'	qRT-PCR of GAPDH
Q529bp-F	5'-CACAGAAGGGACAGAAGT-3'	529bp-based qPCR
Q529bp-R	5'-TCGCCTTCATCTACAGTC-3'	529bp-based qPCR

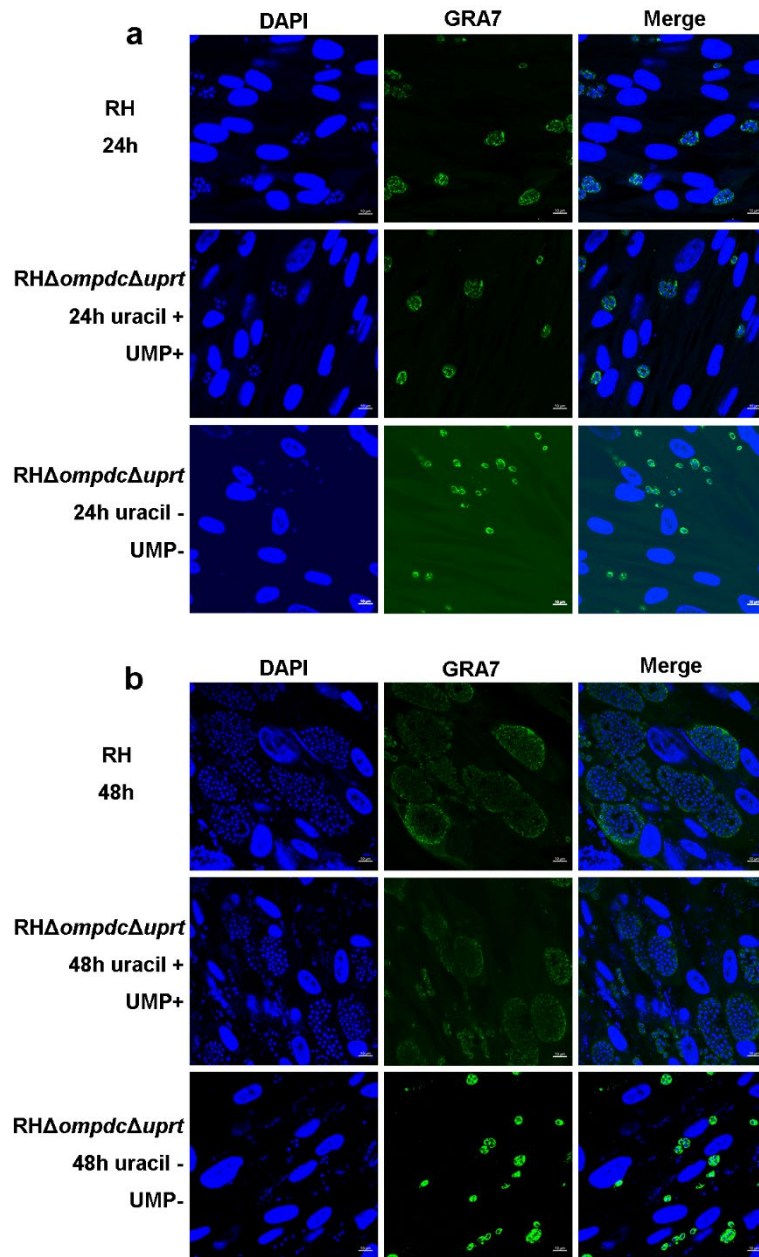
Supplementary Table 2 (Table 2S). This table shows the oocysts in the feces of cats from 1 to 10 days after being infected with RH $\Delta ompdc\Delta uprt$ strain.

Days post infection	1	2	3	4	5	6	7	8	9	10
Blank	-	-	-	-	-	-	-	-	-	-
RH $\Delta ompdc\Delta uprt$	-	-	-	-	-	-	-	-	-	-

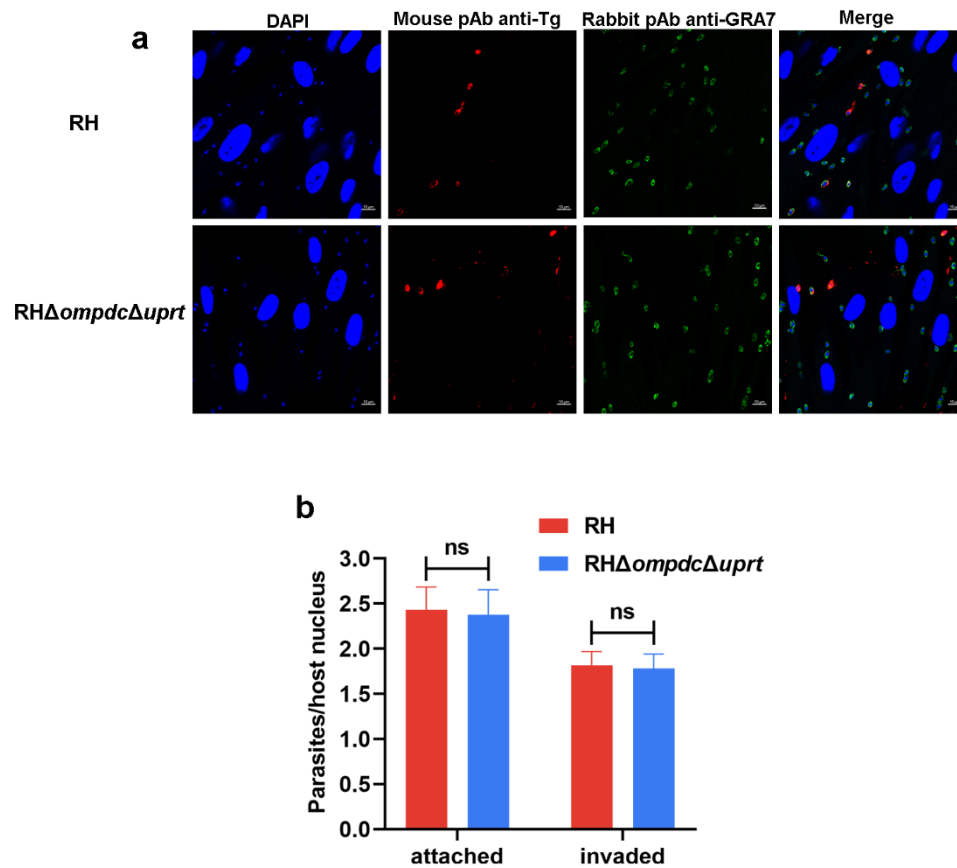
-: no oocysts.



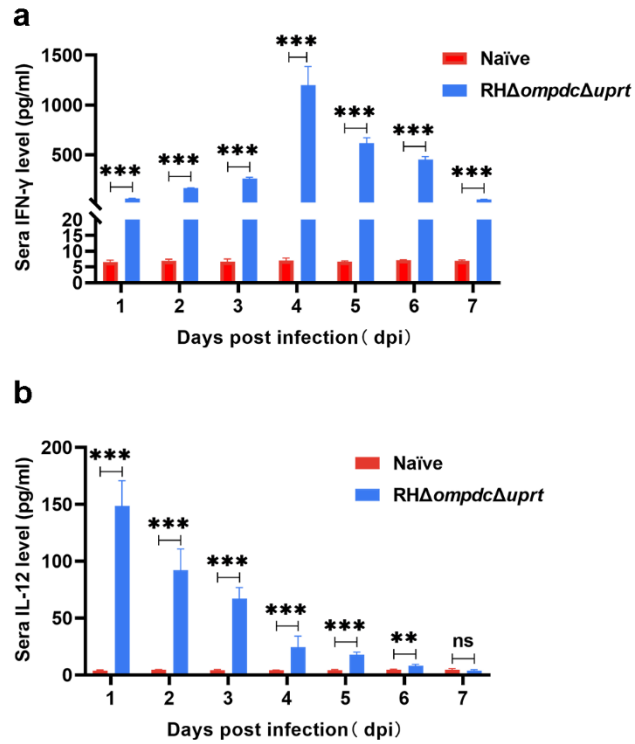
Supplementary Figure 1 (Fig.1S). The diagram of knocking out. **(a)** Schematic illustration of knocking out *uprT* by homologous gene replacement in $RH\Delta ku80\Delta hxgprt$ strain. **(b)** Schematic showing deletion of *hxgprt* gene by insertion of a *uprT* 5'utr-3'utr into *hxgprt* gene. **(c)** Diagram illustrating the deletion of *ompdc* in $RH\Delta uprT\Delta hxgprt$ to make the double mutant $RH\Delta uprT\Delta ompdc::HXGPRT$ by CRISPR/Cas9-mediated homologous gene replacement.



Supplementary Figure 2 (Fig.2S). Intracellular proliferation of OMPDC-UPRT deletion mutant under the fluorescence microscope. **(a)** 24 hours of intracellular proliferation. **(b)** 48 hours of intracellular proliferation.



Supplementary Figure3 (Fig.3S). Invasion and attachment assay for RHΔku80 and RHΔompdcΔuprt strains. **(a)** The invasion and attachment of *T.gondii* RHΔku80 and RHΔompdcΔuprt were evaluated by indirect immunofluorescence assay (IFA). **(b)** the analysis of the invasion and attachment. ns not significant.



Supplementary Figure4 (Fig.4S). The levels of cytokines in the serum of mice injected with RHΔompdcΔuprt. The levels of IFN-γ (**a**) and IL-12 (**b**) were measured by flow cytometer. The results are presented as the means $\pm$ SD. ($n = 5$; $**P < 0.01$, $***P < 0.001$ by Student's t test, ns not significant.)